RESIDUES AND TRACE ELEMENTS

Supercritical Fluid Extraction and Gas Chromatography/Ion Trap Mass Spectrometry of Pentachloronitrobenzene Pesticides in Vegetables

STEVEN J. LEHOTAY and MEDINA A. IBRAHIM
U.S. Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Building 007, Room 224, Beltsville, MD 20705

An analytical approach using supercritical fluid extraction (SFE) followed by gas chromatography/ion trap mass spectrometry (GC/ITMS) was developed for the analysis of the fungicide pentachloronitrobenzene and several analogues in vegetables. The method was tested in the analysis of carrots, potatoes, green beans, celery, and radishes fortified with pentachloronitrobenzene, tetrachloronitrobenzene, pentachloroanisole, pentachlorothioanisole, pentachlorobenzene, hexachlorobenzene, and pentachloroaniline. An incurred carrot sample analyzed by the method was shown to contain hexachlorobenzene at 7 ± 3 ng/g, which agreed with the concentration (8 ± 4 ng/g) determined using a traditional solvent-based method. The SFE method consisted of the following steps: (*1*) homogenizing a 50 g vegetable sample and weighing a 3 g subsample; (*2*) mixing 2 g sorbent (Hydromatrix) with the subsample to absorb moisture and packing a 10 mL extraction vessel; (*3*) extracting with 40 mL CO_2 at 200 atm, 40°C, and a flow rate of 3 mL/min; and (*4*) collecting the extract on a 1 g alumina basic trap at 25°C and flushing with 8 mL isooctane. Collection of the extract on alumina efficiently removed chlorophyll and other matrix interferences. GC/ITMS in the electron-impact mode confirmed and quantitated the analytes at concentrations as low as 1 ng/g.

Food safety is a high priority for the U.S. Department of Agriculture (USDA), the Food and Drug Administration, and other government agencies throughout the world. The list of regulated hazardous contaminants is growing (1), and toxicological data concerning detrimental health effects of pesticides are becoming more alarming (2). Currently, pesticides in food are analyzed by organic solvent extraction methods (3–6), which can be expensive, hazardous, time-consuming, and labor-intensive; require much space and glassware; and generate a large amount of hazardous waste. New methods of multiresidue analysis must be developed and implemented to overcome these problems and to provide more efficient analyses of food contaminants (7).

Supercritical fluid extraction (SFE), an analytical approach developed recently, poses little threat to the environment; saves laboratory space, time, and expense; and lends itself to automation. Many research articles and reviews (8–11) that describe SFE techniques have been published, and commercial SFE instrumentation has become available. Instruments from major manufacturers show few differences in extraction results (12). Despite the great interest in SFE, not many studies describing pesticide extraction from food by this method have been published (12–22).

The major goal of this research was to study the use of SFE for analysis of pesticides in produce. Concurrent development of new cleanup and analysis methods that minimize the use of hazardous solvents was a related goal. Gas chromatography/ion trap mass spectrometry (GC/ITMS) was selected as the detection method. Use of GC/ITMS for multiresidue analysis of pesticides in foods has been reported (23–26). GC/ITMS determines various components in a complex matrix and confirms the presence of regulated contaminants at trace concentrations with one chromatogram.

The soil fungicide pentachloronitrobenzene (PCNB), also known as quintozene, and its co-formulants and metabolites, pentachlorobenzene (PCB), hexachlorobenzene (HCB), tetrachloronitrobenzene (TCNB), pentachloroaniline (PCAL), pentachloroanisole (PCAS), and pentachlorothioanisole (PCTA), were used as analytes for this initial study of SFE for analysis of pesticides in vegetables. PCNB has been found (below tolerance levels) in 0.3% of potatoes tested in California and in 2.5% of green beans tested in Texas and Washington (27). Also, the Environmental Protection Agency (EPA) has emphasized detection of trace concentrations of PCB and HCB because of their high toxicity (28).

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Experimental

Apparatus

(a) *Supercritical fluid extractor.*—Prepmaster (Suprex Corp., Pittsburgh, PA) equipped with manual or automated variable restrictor and a solid sorbent collection system. Results improved dramatically when the manual variable restrictor was replaced with an automated variable restrictor. Instrumental parameters were varied individually to determine the effect of conditions on extraction efficiencies: extraction fluid, CO_2; 10 mL extraction vessel; extraction pressure, 200, 300, or 400 atm; oven temperature, 40, 60, or 80°C; 2 or 15 min static extraction step; CO_2 volume, 30, 40, or 50 mL; flow rate, 1, 2, or 3 mL/min; restrictor temperature, 50°C; alumina, Florisil, or glass beads collection sorbents (1 mL volume); trap temperature, 25°, 0°, or –25°C; isooctane, hexane, or methylene chloride flush solvent; flush temperature, 25°C; up to 10 mL flush volume at 1 mL/min flush rate; and N_2 gas pressure (to blow trap dry between flushes), 80 psi. Optimized extraction conditions for vegetable samples were as follows: 10 mL vessel, 200 atm, 40°C, 2 min static extraction step, 40 mL CO_2, 3 mL/min flow rate, alumina trap collection at 25°C, and 8 mL isooctane flush solvent at 25°C.

(b) *Gas chromatographs.*—Either a Model ITS40 (Finnigan MAT, San Jose, CA) consisting of a Varian 3300/3400 gas chromatograph and CTC A200S autosampler, or a Model 5890 (Hewlett-Packard Co., Avondale, PA) equipped with a Model 7673 Hewlett-Packard autosampler and electron capture detection (ECD) was used for analysis. Typical operating conditions for the GC/ITMS were as follows: 1 µL splitless injection volume; 3 s needle hold time in port before injection; 230°C injection port; split valve state initially on, off at 0.01 min, on at 0.8 min; 11 psig He column head pressure; 100°C initial oven temperature for 1 min, 4°C/min ramp rate to 168°C followed by 8°C/min ramp rate to 200°C, and hold for 3 min (25 min total time); 230°C transfer line temperature; and 210°C detector manifold temperature (20 mTorr). Typical ITMS operating conditions (autotune calibration was performed before each injection sequence) were electron impact mode, 10 µA filament current, and 1850 V electron multiplier tube. Typical operating conditions for the GC–ECD were 1 µL splitless injection volume; 230°C injection port; 0.5 min purge off time; 21 psig He column head pressure; 100°C initial oven temperature for 1 min, 5°C/min ramp rate to 140°C, 3°C/min ramp rate to 170°C, and to 240°C at 15°C/min (23.7 min total time); 300°C ECD temperature; and 18.1 mL/min flow rate of ECD make-up gas (5% CH_4 in Ar).

(c) *Chromatographic columns.*—For GC/ITMS, a (14% cyanopropylphenyl) methylpolysiloxane DB-1701 (30 m × 0.32 mm id, 0.25 µm film thickness), capillary column (J&W Scientific, Folsom, CA) and a 5 m phenyl-methyl deactivated guard column (0.32 mm id) (Restek Corp., Bellefonte, PA) were used. For GC–ECD, a 100% dimethylpolysiloxane SPB-1 (30 m × 0.25 mm id, 0.25 µm film thickness), capillary column (Supelco, Bellefonte, PA) was used.

(d) *Data collection.*—The GC/ITMS used a Magnum version 2.1 software package (provided with the instrument) loaded into a Gateway 2000 computer; the GC–ECD employed a Pascal version Chemstation software loaded into a Hewlett-Packard 300 series computer for data collection and analysis and instrument control. For the ion trap, the data collection range was 127–305 *m/z*, and the scan rate was 7 scans/s.

(e) *Glassware.*—150 mL beakers to weigh and contain samples, stirring rod for mixing, 1 L bottle to contain flush solvent, 12 to 15 mL graduated centrifuge tubes to contain extracts, transfer pipets, and 2 mL autosampler vials for GC analysis. Volumetric flasks and pipets were required for preparation of standard solutions.

(f) *Food processor.*—A food processor (Black & Decker, Shelton, CT) with slicing, shredding, and chopping blades and top-loading chute was used to cut and mix the vegetable samples.

Reagents

(a) *Gases.*—Supercritical fluid chromatography/SFE grade CO_2 with 1800 psi He headspace and dip tube (Air Products, Allentown, PA). Bone-dry grade CO_2 and N_2 were required for cryogenic cooling and drying, respectively, of the solid-sorbent trap.

(b) *Solvents.*—Isooctane (2,2,4-trimethylpentane), hexane (as *n*-hexanes), and methylene chloride (dichloromethane) were pesticide grade (Fisher, Fair Lawn, NJ).

(c) *Solids.*—Hydromatrix (HMX) (Varian, Harbor City, CA) (a pelletized diatomaceous earth material) was sieved (325 mesh) before use to remove fine particles. Alumina (basic, Brockman Activity I, certified grade, Fisher) was sieved (60–120 mesh); before use in the trap, it was oven-dried at 115°C overnight. Florisil (Fisher) was provided in 60–100 mesh particle sizes and was also oven-dried before use. The 80–100 mesh silanized glass beads (Suprex) were used as provided.

(d) *Pesticide standards.*—Pentachloronitrobenzene, pentachloroanisole, 2,3,5,6-tetrachloronitrobenzene, and pentachlorobenzene were obtained from Aldrich Chemical Co. (Milwaukee, WI); pentachlorothioanisole was obtained from EPA (Research Park, NC); and hexachlorobenzene, pentachloroaniline, and 2,3,5,6-tetrachloroaniline were obtained from USDA (Beltsville, MD). All compounds had a purity of more than 98% and were used as received. Individual stock solutions were prepared by weighing 10–12 mg amounts of standards, dissolving the pesticide with hexane or isooctane, and making up to 100 mL in volumetric flasks. A working standard mixture of 10 ng/µL (ppm) was used as a spiking solution and as a stock solution for preparing calibration standards.

Sample Preparation and Analysis

Vegetables used as blank or fortified samples were purchased at a local supermarket. The State of Michigan Department of Agriculture (SMDA) provided an incurred carrot sample (contaminated in the field). A 50 g portion of each vegetable was shredded and mixed in a food processor. For potato, carrot, or radish, a 3 g subsample was weighed in a tared beaker. For

green beans or celery, a 2.5 g subsample was used. To absorb moisture, HMX (2 g for potato, carrot, or radish; 2.5 g for green beans or celery) was mixed with sample with a glass rod. HMX was added to the smaller subsample rather than the entire sample to conserve the sorbent and to ensure the correctness of the weight of vegetable added to the vessel. The mixed samples were packed into 10 mL extraction vessels (a small layer of HMX was added to the top of the vessel to ensure better trapping of moisture). For fortified samples, spiking solution was added to the sample in the vessel to avoid transfer losses. Spiking levels varied between 2 and 250 ng/g, and 3 samples at each concentration were extracted for analysis. For the incurred carrot sample, which arrived precut and frozen, 3 g portions of either thawed or frozen sample were mixed with 2 g HMX and packed into the vessels. A control spike of PCB (100 ng/g) was added to vessels containing frozen samples. The samples were extracted, and 8 mL extract was evaporated to 1 mL (for enhanced detection) in graduated centrifuge tubes under a gentle stream of nitrogen. Then, 100 μL of a 0.5 ng/μL tetrachloroaniline internal standard solution was added. The extracts were mixed with transfer pipets and transferred to autosampler vials for GC analysis. The same solution as the spike solution was diluted to make calibration standards, and the internal standard solution was added to calibration standards in the same ratio as the extracts. Calibration standards at concentrations of 0.005, 0.01, 0.05, 0.1, 0.5, and 1 ng/μL were analyzed with each set of samples.

Calculations

(a) *Limits of detection.*—The $3\sigma_{blank}$/sensitivity approach, where σ_{blank} is the standard deviation of the blank signal, was used to calculate limits of detection (LODs). For GC–ECD data, LODs were calculated by dividing 3/5 of the peak-to-peak baseline noise (in picoamperes) near the retention time (t_r) of the analyte by the slope of the peak height (in picoamperes) calibration curve. For GC/ITMS data, the average noise in the 60 s retention windows of chosen quantitation masses was calculated from software-reported signal-to-noise ratios for calibration standards. LODs were calculated by multiplying these noise levels by 3 and then dividing by the slope of the calibration curve generated from peak height data. External standard calibration curves corrected for dilution by addition of internal standard solution were used for LOD calculations.

(b) *Sample concentrations.*—Integrated peak area data vs internal standard were used for quantitation. Either a spreadsheet program or the software program from the instruments was used. Because calibration standards were treated in the same manner as extracts, the change in concentration caused by addition of internal standard was disregarded. The calculated concentrations of analytes in extracts were corrected for concentration during extraction and solvent evaporation steps to obtain the concentrations of analytes in samples. Recoveries (%) were calculated by dividing the experimental concentrations by the expected fortified concentrations (×100).

Results and Discussion

This SFE procedure was developed in several steps. Analytical GC conditions were optimized using available columns for most rapid separation of the components. Detection methods were compared. SFE experiments were divided into 3 categories: sampling, extraction, and collection–cleanup. Sampling was controlled by vessel size, desired levels of analyte detection, and other practical considerations (time of analysis, amount of water in sample). Parameter effects on extraction recoveries were investigated by altering one SFE parameter at a time. Collection–cleanup was investigated by spiking the analyte mixture on different solid sorbents and flushing with different solvents. The choice of sorbent–solvent pair was based on whether chlorophyll was separated from the analytes and how much solvent was required to rinse the trap.

Analysis

Polychlorinated pesticides were chosen for this study; thus, GC–ECD was believed to be the most sensitive analytic method. Optimum instrumental parameters were determined to achieve the most rapid fully resolved separation of analytes with the column available. Calibration standards were analyzed to calculate LODs. The same examination was made with the GC/ITMS instrument. Table 1 presents results of GC–ECD and GC/ITMS analyses. LODs from GC–ECD were 3–15 times lower than LODs from GC/ITMS, but the detection capability of GC/ITMS was still sufficient for trace pesticide analysis. For analysis of sample extracts, an important feature was the ability of ITMS to separate the analyte spectrum from matrix interferences on the basis of quantitation masses. Confirmation of contaminants at trace concentrations is a primary function of regulatory pesticide residue analysis. Quantitation and confirmation of the presence of pesticides in a sample with a single injection was an advantage of GC/ITMS. Chemical ionization (CI) is more commonly used for analysis of pesticide residues in food because of severe matrix interferences (23–26), but electron impact (EI) ionization allows better chemical identification and structure elucidation. EI gave lower LODs for polychlorinated analytes than did CI. A notable feature of SFE was that extracts were adequately free of matrix interferences for GC/ITMS analysis in EI mode.

Sampling

(a) *Sample size.*—Available volumes of SFE vessel were 0.5, 1, 3, 5, 8, 10, 30, and 50 mL. For the 30 and 50 mL vessels, extraction times were >1 h, >100 g CO_2 was required per extraction, and matrix effects were more pronounced compared with the smaller vessels. The detection limits of the method did not require the larger sample size, because analyte at 1 ng/g is detected in a 3 g sample without difficulty. Detection of pesticide at 1 ng/g in a sample is adequate for most pesticides in foods as denoted by regulatory tolerance levels (29). The 10 mL vessel was chosen because it accommodates more than 3 g vegetable material, and its use does not lead to a long extraction time.

Table 1. GC–ECD and GC/ITMS results[a]

Analyte	GC–ECD		GC/ITMS		
	t_r, min	LOD, pg	t_r, min	Quantitation masses	LOD, pg
PCB	12.4	0.28	12.1	248 + 250 + 252	0.69
TCNB	14.2	0.21	15.9	203 + 215 + 261	3.3
HCB	17.8	0.14	17.0	282 + 284 + 286	0.77
PCAS	18.1	0.10	18.0	237 + 265 + 280	1.3
PCNB	19.2	0.24	20.0	237 + 265 + 295	2.9
PCAL	21.0	0.19	22.3	263 + 265 + 267	0.79
PCTA	ND	ND	22.7	294 + 296 + 298	1.8

[a] LOD, limit of detection. ND, not done.

Whether a 3 g subsample was representative was determined. Traditional solvent extraction methods use sample sizes of 50–100 g, but such sample sizes were needed because of poor detection sensitivity (4, 5). For most commodities, subsampling from a well-mixed larger sample yields a homogeneous sample. The reproducibility of results from analysis of subsamples of incurred carrot supports this contention.

(b) *Water in the sample.*—Because of concerns with restrictor and trap collection–elution operations, the SFE instrument could not withstand water flowing through the system. The vegetables contained 70–90% water, which had to be removed or absorbed. Removal of water from string beans by freeze-drying was tested, but eight 50 g samples required over 24 h to dry, and the concentration of matrix interferences overwhelmed the collection–cleanup approach. Freeze-drying results in losses of analyte through volatilization, an effect not observed in previous SFE experiments. Volatile chemicals are more easily lost during lyophilization. Earlier SFE studies showed that a completely dry sample gave slightly lower analyte recoveries than do samples containing water (absorbed on HMX). Therefore, removal of water by absorption was better than drying, because detection sensitivity was not an issue.

HMX, a commercially available diatomaceous earth, has been investigated for SFE purposes (13) and was chosen for use in this study. Experiments were conducted to determine the amount of HMX required to remove water. The material absorbs twice its weight in water at room conditions, but when the water:HMX ratio was 2:1 and extraction was done at elevated temperature and pressure, water was observed in the system. However, HMX holds its own weight of water at SFE conditions, so a 1:1 water:HMX ratio was used for all extractions. Because potatoes, carrots, and radishes were ca ⅔ water (in samples from a grocery store), 2 g HMX was used for 3 g sample; for green beans and celery, which were 90% water, a 1:1 ratio of sample:HMX was used. These amounts were reasonable for packing the 10 mL vessel with ease.

Extraction Conditions

(a) *Pressure and temperature.*—Extraction conditions at a range of pressures and temperatures were examined. Green beans samples mixed with HMX (1:1) were loaded into vessels and spiked at 250 ng/g with a 4-component analyte mixture. Other extraction parameters were 2 min static time (to ensure equilibration at set conditions); 1 mL/min flow rate; 40 mL CO_2 volume; and collection on 1 mL glass beads at 25°C, which were flushed with 4 mL hexane. The extract was cleaned up by washing with 10 mL hexane through a 1 mL alumina column. Figure 1 presents results for this experiment. Recoveries for triplicate extractions were 80–120% at all conditions except at 300 atm and 60°C. The lower recovery and larger variability (70 ± 35%) at these conditions were believed to be in error (perhaps carryover occurred and produced >100% recovery at 300 atm and 80°C), but the study was not repeated. All conditions tested gave acceptable recoveries (80%), and with further optimization of parameters, 100% recoveries were considered obtainable. Highest recoveries were obtained at 300 atm and 80°C (CO_2 density of 0.75 g/mL), and these conditions were chosen for further study.

The results shown in Figure 1 were from extractions performed early in the study. In subsequent extractions, recoveries of analytes were nearly always proportional to each other, and if one analyte deviated from the others, the deviation was associated with analysis. In these cases, analysis was redone with different quantitation masses. Also, significant differences in recoveries from tests with spiked HMX or sample matrix were not observed. As the study progressed, reproducibility of re-

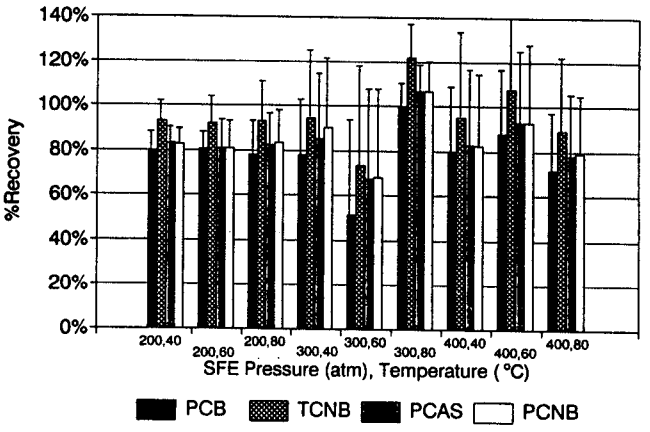

Figure 1. Results for spiked (4 components, 250 ng/g) green beans at different extraction pressures and temperatures.

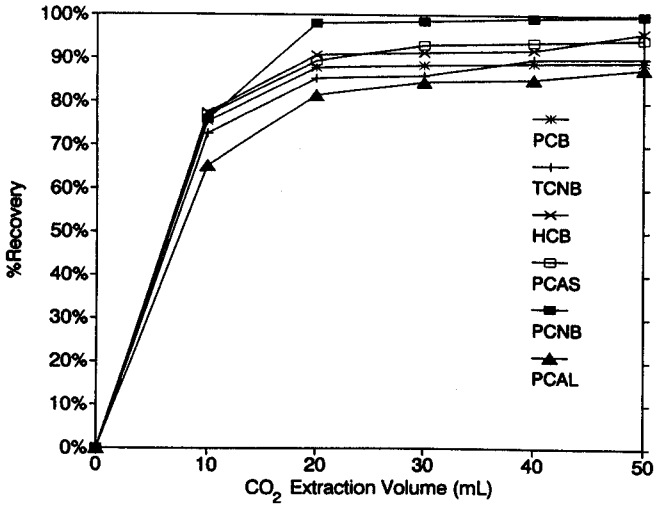

Figure 2. Recoveries from spiked (100 ng/g) 1:1 Hydromatrix:water versus CO_2 extraction volume.

coveries improved (from 30% relative standard deviation [RSD] to 5% RSD) because of instrument modifications and optimization of parameters. Improvement in results were most dramatic when the manual variable restrictor on the SFE instrument was replaced with an automated variable restrictor.

(**b**) *Amount of CO_2.*—To determine the required amount of CO_2 for complete extraction, 1:1 Hydromatix:water test samples were loaded into 10 mL vessels, spiked, and extracted at 300 atm, 80°C, 2 min static time, and 1 mL/min CO_2. The extract was collected as before, but extraction was stopped after every 10 mL CO_2, and the trap was flushed with 4 mL hexane. The 5 flushes were collected separately and analyzed. Figure 2 displays the effect of CO_2 volume on recovery, and on the basis of these results, a CO_2 extraction volume of 40 mL (30 g) was used subsequently.

(**c**) *Static time.*—Static time at the beginning of the run was increased from 2 to 15 min, and the experiment to determine the amount of CO_2 required for extraction was repeated. The volume of CO_2 required for highest recovery had not changed significantly, and the 2 min static time was retained. A longer static time could have made a difference if a solvent modifier, such as methanol, was added to the CO_2 (9–11), but it was decided not to use solvent modifiers if recoveries were acceptable without them.

(**d**) *Flow rate.*—All parameters were held constant at the previously determined conditions, except CO_2 flow rate. Flow rate was set at 1, 2, or 3 mL/min with the automated variable restrictor. As shown in Figure 3, the various flow rates gave similar results. To save time, the highest flow rate that maintains reproducible high recoveries should be used. A flow rate faster than 3 mL/min may yield satisfactory results in a shorter extraction time, but the automated restrictor could not stably maintain flows above 3 mL/min. With the 2 min static time, SFE took 15.3 min per sample at the 3 mL/min flow rate.

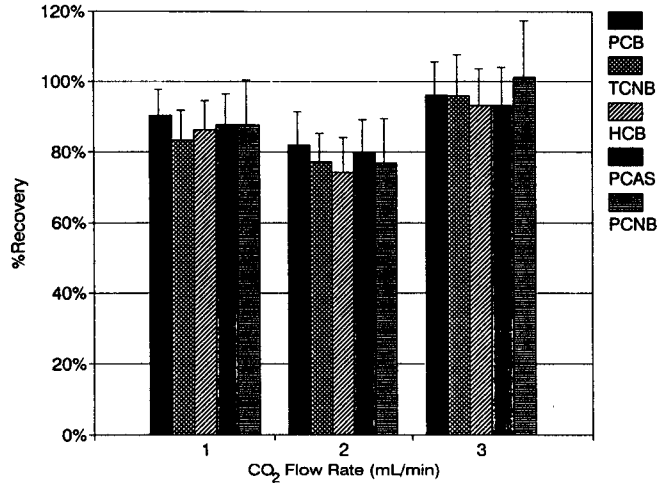

Figure 3. Recoveries of spiked samples at different flow rates.

Collection–Cleanup

(**a**) *Collection temperature.*—The effect of trap temperature during collection on analyte recoveries was studied. Trap temperature of the glass beads was varied from 25°, to 0°, and to –25°C, and samples were extracted under the same conditions outlined earlier. Recoveries at the various trap temperatures were not significantly different. The same effect was observed with alumina in the trap. A trap temperature of 25°C was selected because it required less time and less CO_2 to maintain. Also, the chance of plugging the restrictor or trap with ice was lower at the higher trap temperature.

(**b**) *Solid sorbent.*—When extracting green beans, the glass beads successfully trapped analytes, but chlorophyll was eluted also by hexane from the trap. Other solid sorbents (alumina and Florisil) and flush solvents (isooctane and methylene chloride) were tested. It was hoped that by placing the cleanup sorbent in the trap the extraction–cleanup could be automated.

(**c**) *Cleanup.*—Different sorbent–solvents were studied by filling the 1 mL trap with activated (baked) materials. After the traps were rinsed with solvent, a blank green beans extract was spiked with analytes and added to the top of the trap column. The trap was flushed with solvent, and 1 mL fractions were collected and analyzed. Even after 10 mL of solvent had been used, no pesticide was completely eluted from Florisil. Figure 4 presents results for the isooctane and hexane flushes of the alumina trap. A 6 mL isooctane flush eluted 100% of the analytes from alumina, whereas 8 mL hexane eluted all of the pesticides; chlorophyll remained on the alumina in both cases. Isooctane was selected for subsequent extractions, and an 8 mL flush volume was used. More than 20 extractions of green beans could be performed before chlorophyll was noticeable in the extracts.

Flush temperature and flow rate were always 25°C and 1 mL/min, respectively, during extractions. The effects of temperature and flow rate were not determined, and perhaps some combination of increased temperature and/or decreased flow rate may save time and volume of solvent for flushing and may improve the reproducibility of complete removal of PCAL (Table 2) from alumina.

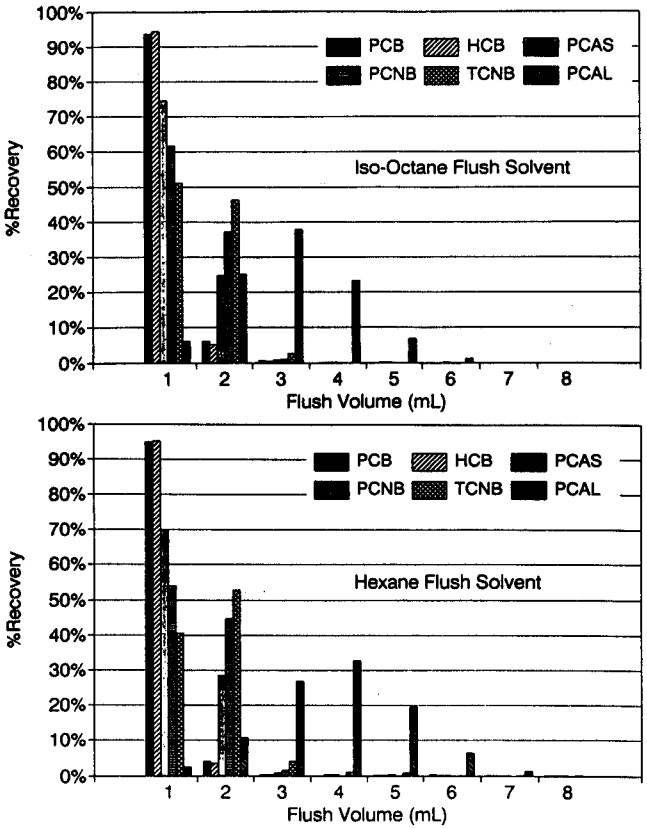

Figure 4. Comparison of flushing volume required to elute analytes from the 1 mL alumina trap with isooctane and hexane.

SFE of Fortified and Incurred Samples

The method was tested by analyzing spiked and incurred samples. Fortified green beans, potatoes, celery, and radishes were extracted without difficulty at the chosen conditions; however, carrot extracts were yellow despite alumina cleanup.

(a) *Xanthophyll in carrots.*—Examination of the alumina afterwards revealed an orange color at the top of the column, which suggested that β-carotene was not eluted. The UV–visible absorbance spectrum of the extract showed peaks corresponding to the spectrum of xanthophyll, or β-carotene-3,3′-diol (30). The extracts could be analyzed by GC/ITMS, but for long-term regulatory analyses, removal of the matrix component was investigated. As shown in Figure 1, other extraction conditions gave satisfactory results, and rather than alter the cleanup process to remove xanthophyll, the pesticides were more selectively extracted at different SFE conditions. The concentration of xanthophyll in the extract was reduced by extracting at 200 atm and 40°C (CO_2 density, 0.85 g/mL).

Table 3. HCB in thawed and frozen subsamples of incurred carrot samples

	HCB, ng/g	
Replicate	Thawed subsample	Frozen subsample
1	2.02	10.1
2	1.78	6.3
3	1.93	4.6
4	1.75	ND[a]
Average ± std. dev.	1.85 ± 0.13	7.0 ± 2.8

[a] ND, not done.

(b) *Fortified samples.*—Table 2 lists average results and standard deviations of recoveries from fortified samples of different produce. Results for extractions at different SFE conditions, at different fortification levels, and analysis by GC/ITMS or GC–ECD are included. Results at low fortification levels were similar to those at higher fortification levels. Results of initial experiments at nonoptimized conditions are included in Table 2, because they were considered relevant to the overall methodology, and day-to-day instrumental effects are represented in the results. In all, 111 extractions of PCB, TCNB, PCAS, and PCNB; 84 extractions of HCB and PCAL; and 33 extractions of PCTA in fortified vegetables were performed. Overall recoveries (%) ± standard deviations were 89 ± 15 for PCB, 92 ± 17 for TCNB, 88 ± 13 for HCB, 90 ± 16 for PCAS, 91 ± 21 for PCNB, 89 ± 32 for PCAL, and 93 ± 18 for PCTA. For all analytes average recovery was 90%, with 21% RSD. In some experiments (as evidenced by PCTA results of

Table 2. Recovery of analytes from fortified vegetables

	Recovery (%) ± standard deviation				
Analyte	Carrot (17)[a]	Celery (16)[a]	Green beans (45)[a]	Potato (23)[a]	Radish (10)[a]
PCB	100 ± 12	87 ± 15	84 ± 18	92 ± 15	94 ± 6
TCNB	79 ± 8	78 ± 14	94 ± 22	92 ± 17	127 ± 19
HCB	99 ± 13	78 ± 15	86 ± 12[b]	90 ± 16	82 ± 8
PCAS	101 ± 14	77 ± 16	91 ± 20	92 ± 14	82 ± 9
PCNB	100 ± 18	82 ± 17	86 ± 24	92 ± 18	112 ± 24
PCAL	89 ± 38	78 ± 32	98 ± 22[b]	89 ± 36	88 ± 28
PCTA	106 ± 25	ND[c]	99 ± 4[d]	99 ± 3[d]	68 ± 14

[a] Numbers in parentheses are replicate extractions.
[b] HCB and PCAL were spiked in 18 samples for green bean.
[c] ND, not done.
[d] PCTA was spiked in 3 samples for green bean and potato.

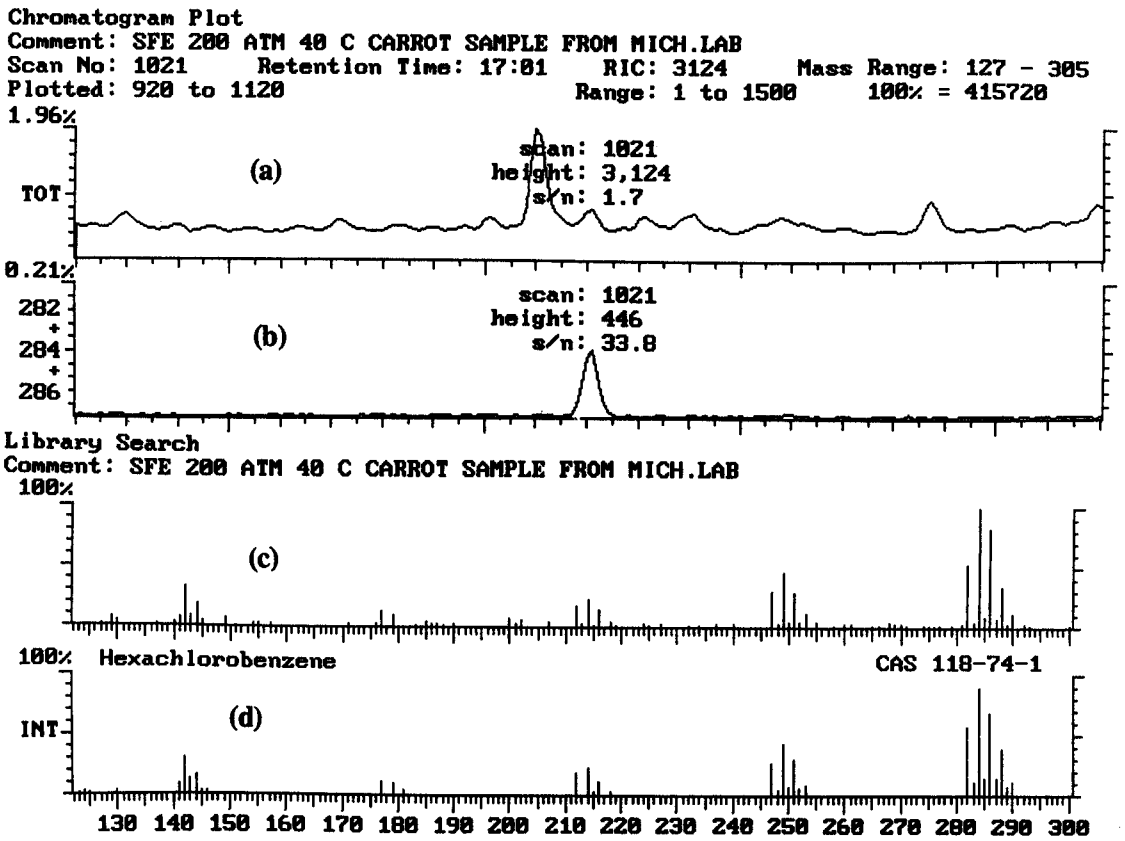

Figure 5. Analysis of HCB in incurred carrot extract: (a) total-ion chromatogram, (b) chromatogram of HCB quantitation masses, (c) background-corrected mass spectrum of the peak, and (d) search library identification of HCB.

green beans and potato), 100% recoveries were obtained with less than 5% RSD, but 10–20% RSD was more common. In general, potato samples gave best results, because of the sample texture and fewer matrix interferants. However, recoveries and variability were more dependent on instrumental operation than sample matrix. The difficulty of maintaining optimal instrumental conditions (for both SFE and GC/ITMS) was the greatest disadvantage of the method.

Recoveries of PCAL from fortified vegetables gave the largest variation (20–40% RSD). Because PCAL eluted last from alumina (Figure 4), the higher variability in results was most likely due to an irreproducible isooctane flush volume required to completely remove PCAL from the alumina trap. Use of a larger isooctane flush volume should improve PCAL variability but at greater cost of time and amount of solvent used.

(c) *Incurred carrots.*—The carrot sample was previously analyzed twice by SMDA, using the procedure reported by Luke et al. (3) and GC/electrolytic conductivity detection. Hexachlorobenzene (HCB) was found at a concentration of 8 ± 4 ng/g (between the 4 ng/g LOD and 12 ng/g limit of quantitation for the method). For SFE, the carrots were thawed, and 4 subsamples were extracted and analyzed. In a repetitive study, triplicate subsamples were kept frozen as they were mixed with HMX and packed into the extraction vessels. As shown in Table 3, use of frozen subsampling gave an HCB concentration (7 ± 3 ng/g) that was in better agreement with the HCB concentration determined by SMDA (the result was 1.8 ± 0.1, lower than the LOD of their method). In a further refinement of the method, PCB was added at 100 ng/g to the frozen sample in the vessel to serve as a matrix spike to ensure recovery (PCB recovery was $110 \pm 6\%$). The recovery of one compound was nearly always similar to those of others in any given extraction.

The thawed carrot gave a lower HCB concentration than the frozen sample due to increased volatilization of HCB at room temperature. On the basis of these results, frozen samples should be used in sample preparation for SFE.

HCB in incurred carrot was accurately and precisely quantitated at concentrations less than 2 ng/g by SFE and GC/ITMS; the analyte was confirmed with ITMS. Figure 5 shows the total-ion chromatogram of an incurred carrot extract at the t_r of HCB, the quantitation peak, the mass spectrum (background subtracted), and the library spectrum for HCB. The spectrum correlation was 87% for HCB, the same correlation as the HCB standard vs the EI mass spectrum in the search library. Analysis of the incurred sample illustrated the analytical power of GC/ITMS.

Conclusions

This study illustrates the use of SFE and GC/ITMS to extract, analyze, and confirm various pesticides at trace concentrations in food. PCNB and analogues were extracted and analyzed, and the results demonstrate the multiresidue capability of the method. Because of the enhanced selectivity of SFE and the smaller sample size required, matrix interferences were minimized. An easy, automated SFE–cleanup was possible with extract collection on a solid-sorbent trap. SFE generates a smaller quantity of hazardous waste than many solvent-based extractions. Thus, expense and hazards to workers and the environment are lower. Because SFE is automated, time and labor of laboratory personnel are also decreased. GC/ITMS saves time because quantitation and confirmation is accomplished in analysis. SFE produced vegetable extracts sufficiently clean for mass spectrometric detection in the EI mode.

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